

Invited Review

How do UV Photomorphogenic Responses Confer Water Stress Tolerance?^{¶†}

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ABSTRACT

Although ultraviolet-B (UV-B) radiation is potentially harmful, it is an important component of terrestrial radiation to which plants have been exposed since invading land. Since then, plants have evolved mechanisms to avoid and repair UV radiation damage; therefore, it is not surprising that photomorphogenic responses to UV-B are often assumed to be adaptations to harmful radiation. This presupposes that the function of the observed responses is to prevent UV damage. It has been hypothesized that, as with blue light, UV-B provides a signal important for normal plant development and might be perceived within developing plants through nondestructive processes, perhaps through UV-specific signal perception mechanisms. UV signal perception can lead to photomorphogenic responses that may confer adaptive advantages under conditions associated with high-light environments, such as water stress. Plant responses to UV radiation in this regard include changes in leaf area, leaf thickness, stomatal density, photosynthetic pigment production and altered stem elongation and branching patterns. Such responses may lead to altered transpiration rates and water-use efficiencies. For example, we found that the cumulative effect of ambient UV-B radiation upon stomatal density and conductance can lead to altered water-use efficiencies. In field settings, UV might more properly be viewed as a photomorphogenic signal than as a stressor. Hence, it might be insufficient to attempt to fully evaluate the adaptive roles of plant responses to UV-B cues upon stress tolerance by the simultaneous application of UV and drought stress during development. We propose that

rather than examining a plant's response to combinations of stressors one might also examine how a plant's response to UV induces tolerance to subsequently applied stresses.

INTRODUCTION

It is generally accepted that ultraviolet-B (UV-B, 280–315 nm) affects both the physiology (1) and the development (2) of plants. Although the UV-B wave band comprises a small fraction of the total terrestrial solar spectral irradiance, UV-B radiation elicits disproportionately large responses from plants. The interest in UV-B effects on plants is not limited to stress and yield effects on crop plants (3) but includes changes in ecosystem composition (4–6).

Work on the effects of UV-B on plants continues to be summarized in periodic reviews (1–8). In general, plant responses to UV-B can be arbitrarily divided into two classes, photomorphogenic and stress responses. Stress responses are characterized by damage leading to reductions in metabolism and growth and development that result from an applied or increased level of a given stressor as compared with a plant's "normal" growing conditions (9). An exception to this view is the case of crop plants, where stress effects have been extended to include decreases in crop yield, whether they are the result of stress or photomorphogenic responses (3). Photomorphogenic responses resulting in altered architecture are usually thought to be adaptive responses of plant development to the light microenvironment or perhaps more accurately the radiation microenvironment through nondamaging signal perception pathways. Although chemical composition might not generally constitute a classical morphological characteristic *in stricta*, the specific case of secondary phenolic accumulation in response to UV-B might be considered a photomorphogenic response. This is especially true regarding plant responses to UV-B radiation, such as flavonoid induction, that have been described as photomorphogenic responses (10).

Photomorphogenic UV-B responses are typically assumed to be adaptations to radiation stress (5). This presupposes that the primary function of the observed responses is to prevent UV damage. Furthermore, it has been shown that UV-B provides a signal important for normal plant development (2). Experiments in which plants were grown in UV-B-deficient photoregimes showed that UV-B radiation is required for normal leaf

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Abbreviations: HCA, hydroxycinnamic acid derivatives; NP, Natural Products reagent; UV-B, ultraviolet-B.

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development (11). Cotyledon curling in *Brassica napus* (L.) has been shown to be a UV-B-specific response (12). Inhibition of hypocotyl expansion in tomato (*Lycopersicon esculentum* Mill.) involves a UV-B-specific nonphytochrome photoreceptor activated with extremely low levels of radiation, on the order of $1 \mu\text{mol m}^{-2} \text{s}^{-1}$ (13). It has been hypothesized that low-level UV radiation may act as a signal that suppresses hypocotyl elongation, allowing tomato seedlings to develop a protective pigmentation before emerging into direct sunlight (14). Although such reasoning provides a rationale for the adaptive significance of similar responses in other plants (15,16), it fails to explain positive phototropism induced by UV-B (17, and references therein). Other photomorphogenic UV-B responses, such as leaf thickening and increased leaf pigmentation, have become generally accepted as adaptive responses to UV radiation (10,18,19). This complicates studies because an adaptive or defensive response may mask or eliminate the otherwise measurable plant stress. Therefore, it is important to differentiate between responses having a defensive role and those that might have little to do with increasing tolerance to UV-B.

We hypothesize that some plant responses to UV-B might additionally function to protect plants from environmental stresses typically associated with high ambient UV-B levels rather than from the damaging effects of UV-B itself. Specifically, UV-B may act as high-light signal-inducing photomorphogenic responses that confer tolerance to environmental and physiological conditions associated with high-light environments, such as oxidative stress, photoinhibition and drought.

It should be borne in mind that the relevance of the discussion in this study is not limited to the UV-B wave band. Although the existence of discreet UV-B-specific photoreceptors has yet to be conclusively demonstrated, evidence has continued to accumulate that in some cases similar or identical photoreceptors are involved, allowing plants to similarly respond to UV-B, ultraviolet-A and blue wavelengths both architecturally and chemically (10,13,20). Moreover, it has been suggested that some of the reported blue-UV effects may have resulted from phytochrome instead of blue-UV photoreceptor action (21).

DOES UV AFFECT DROUGHT TOLERANCE?

Photomorphogenic responses adaptive to high-light environments are well known; examples of these can be cited at the whole-plant, organ, tissue and cellular levels of organization and development. Inhibition of shade avoidance characteristics, such as stem and petiole elongation, has been shown to be strongly influenced by the phytochrome photostationary state (22). Although such plant responses are also known to result from UV and blue irradiation, they are not typically known as shade avoidance responses (19). Regardless of the sensory pathway through which stem shortening is elicited, the morphogenic effect is important regarding crop canopy dynamics and light competition between developing plants. Epidermal characteristics such as stomatal density are affected by light intensity. Stomata, plant epidermal pores, have a fundamental role in controlling photosynthesis and transpiration and, hence, water-use efficiency, drought sensitivity and yield. Experiments with garden beans showed that stomatal density is influenced by white light levels (23). Results of an early survey of several species are consistent with a strong influence of the light environment on stomatal development (24).

Empirical evidence substantiates the premise, at least in part, that UV-B radiation may affect drought tolerance. In field-grown soybean [*Glycine max* (L.) Merr.] and cowpea [*Vigna unguiculata* (L.) Walp.], drought and UV-B stress effects were not additive, suggesting that either UV-B increases drought tolerance or vice versa (25–27). Instantaneous water-use efficiency has been shown to be affected by supplemental UV-B in soybean and rice (*Oryza sativa* L.) (28,29). Ambient UV radiation at levels currently encountered in field settings has been shown to modify leaf morphology and gas exchange in *Populus trichocarpa* Torr. & Gray (30). Recent work has demonstrated that UV-B radiation affects stomatal development in pea (*Pisum sativum* L.), although this was compensated for by leaf area reduction (31). Further experiments suggested that UV-B radiation affects conductance by direct action on guard cells rather than a cumulative effect on developmental processes (32). Nevertheless, a causal relationship between photomorphogenic responses and gas exchange parameters, which leads to enhanced drought tolerance, may exist in other plants (33). Clear demonstrations of the specific mechanisms behind increased drought tolerance through specific UV-B-mediated photomorphogenic processes remain sparse.

HOW UV MAY AFFECT DROUGHT TOLERANCE

Of specific interest in this study is the identification of those photomorphogenic responses that are elicited by UV-B, expression of which could potentially result in increased tolerance to drought stress (Table 1). Although the potential physiological ramifications of UV-B responses such as altered stomatal density are fairly obvious, it may be difficult to identify how other developmental responses affect drought tolerance. This is partly due to the myriad UV-B responses reported but might also be because many such developmental responses affect stress tolerance only indirectly. For example, it is unknown how UV-induced epidermal flavonoid accumulation, long thought to function as UV-B screens protecting underlying photosynthetic mesophyll tissues, might measurably affect drought tolerance in field settings.

In principle, drought tolerance could result from adaptive strategies other than limiting water loss. For example, the relative enhancement of root growth by UV-B such as that induced by blue light in soybean might increase water availability (34). In this study, we have focused on mechanisms by which UV-B might induce drought tolerance by reducing transpirational water loss while maintaining or increasing the photosynthetic rate.

Decreased stem elongation

Although largely genetically determined, stem elongation is greatly influenced by light. Conditions associated with high light levels such as enhancement of the blue component of the total solar flux or altered red–far red ratios inhibit stem elongation (22,35). UV-B inhibition of stem elongation is thought to reduce the potentially harmful radiation reaching into a canopy and so is thought to be an adaptive response to radiation stress (16,19). But such an attenuation is not wavelength specific and also reduces the total solar load delivered to the leaf, resulting in lower leaf temperatures and reduced water loss. Internode shortening also impedes air movement through a canopy or an isolated plant. Such a reduction in air velocity increases leaf boundary layer resistance, resulting in lowered transpiration rates (36).

Increased leaf thickness

UV-B often reduces leaf area and increases leaf thickness (3). It has been proposed that this might allow the upper mesophyll to participate in screening UV before it can reach the lower palisade and spongy mesophyll. With this strategy, the photosynthetic capacity of the upper mesophyll is sacrificed to some extent so that competence of the deeper tissues is maintained (19). This could lead to loss of a substantial proportion of the total photosynthetic capacity of a thin leaf, but increased leaf thickness effectively increases the amount of mesophyll beneath a unit area of leaf. In thick leaves there is more photosynthetic capacity under a given gas exchange interface. Leaf area reduction further limits potential transpiration loss. Hence, leaf thickening is adaptive to both UV-B and drought stress and is observed in soybean grown under both water stress and enhanced UV-B regimes (25). For such reasons factorial experimental designs in which plants develop under combinations of stressors are often unable to resolve potential causal relationships between UV photomorphogenesis and increased drought tolerance.

Increased trichome density

Trichomes contain high concentrations of secondary phenols and efficiently absorb UV-B radiation (37). However, trichomes absorb and reflect other wave bands of solar radiation as well and can appreciably shade the leaf surface, reducing the foliar heat load and reducing water loss. Increasing the trichome density increases the thickness and persistence of the boundary layer in leaves, reducing water loss (36). Trichome density has been shown to increase in tobacco in response to increased UV radiation (38). In principle, increased trichome density might result from either induction of trichome differentiation or a reduction in leaf surface area. Increased trichome density could result from UV-induced reduction of leaf area, even though trichome numbers per leaf remained constant. Mild drought stress also limits water availability to drive leaf expansion, resulting in smaller leaves. Therefore, developmental responses that confer drought stress tolerance could result from either UV-B or water stress. This again suggests that to effectively separate similar responses arising from different stressors, one might allow plants to develop under different UV regimes and investigate the response to subsequently applied water stress. Interestingly, a functional link between UV induction and drought induction of trichome differentiation may exist at the molecular level. In cotton, transcription factors implicated in controlling trichome development have been shown to belong to a family of drought-inducible transcription factors. These transcription factors show similarity to those implicated in phenylpropanoid synthesis (39). This indicates that portions of the signal transduction pathways involved in expression of UV and drought responses are either similar or shared in cotton.

Increased secondary phenolic metabolism

It is well known that UV-B increases soluble phenolic content in many of the plants investigated to date. But how can this feature increase plant tolerance to water stress? The flavonoids are transparent to photosynthetically active radiation, exhibit high molar absorptivities in the UV and are restricted to the epidermis in dicotyledonous plants, including soybean (40,41). Given these characteristics and the virtual ubiquity of secondary phenolic compound expression in tracheophytes, it is generally accepted that

Table 1. Potential advantages conferred by selected photomorphogenic responses to UV-B. Functional significance regarding both solar UV and water stress tolerance is shown

Response to UV-B	UV tolerance	Drought tolerance
Reduced height, increased branching	Canopy shading of UV-B	Reduced leaf radiation load, air velocity lower within canopy
Cuticle and wax, composition and content	Increased UV reflectance and absorption	Decreased cuticular water loss
Increased trichome density	Increased UV-B absorption	Increased boundary layer resistance, decreased solar heat loading
Decreased stomatal density	Unknown	Decreased stomatal transpiration
Increased secondary phenolic accumulation	Absorption of UV-B	Maintenance of stomatal function via UV screening
Increased leaf thickness	Mesophyll protected by increased radiation path length	Increased photosynthetic capacity under unit leaf area

a primary advantage conferred by these compounds, especially flavonoids, is to screen UV before it can reach underlying photosynthetically active tissues. In addition, it has been shown that hydroxycinnamic acid derivatives (HCA) may be as important or even more important with regard to UV screening than flavonoids in some instances (42,43). Because expression of soluble vacuolar HCA is not restricted to the epidermis, the possibility exists that tissues beneath the epidermis are adapted to participate in UV screening (19,44).

However, guard cells exist on the frontier of the leaf without the protection of overlying epidermal pavement cells. They are metabolically active and extremely sensitive and responsive to rapid changes in the microenvironment about the leaf. A direct physiological effect upon the stoma by UV-B radiation in *P. sativum* L. has been demonstrated (32). Because varying stomatal aperture is the major mechanism by which plants control water loss, maintaining stomatal responsiveness is critical for plants. Given their location, sensitivity and critical physiological role, screening of potentially harmful radiation may represent a unique problem in these cells.

In *Vicia faba* L., 60% of the epidermal flavonoids are restricted to the guard cells (45). It has been suggested that the function of flavonoids in guard cells may be to protect the organelles (especially chloroplasts) from UV damage, ensuring they remain functional (J. McClure, personal communication).

Results from our studies of flavonoid localization in soybean support this hypothesis. Freshly sectioned soybean leaf samples were either fumed with NH₃ or treated with Natural Products reagent (NP, 2-aminoethyl diphenylborinate) to enhance secondary phenolic fluorescence and then viewed by UV epifluorescence microscopy (46,47). Attempts to make cross sections typically resulted in ruptured epidermal cells because the tilelike epidermal cells that comprise the "pavement" of the epidermis were much wider than the thickness of the cross sections. Because of the resulting loss of vacuolar contents, results are shown for both leaf

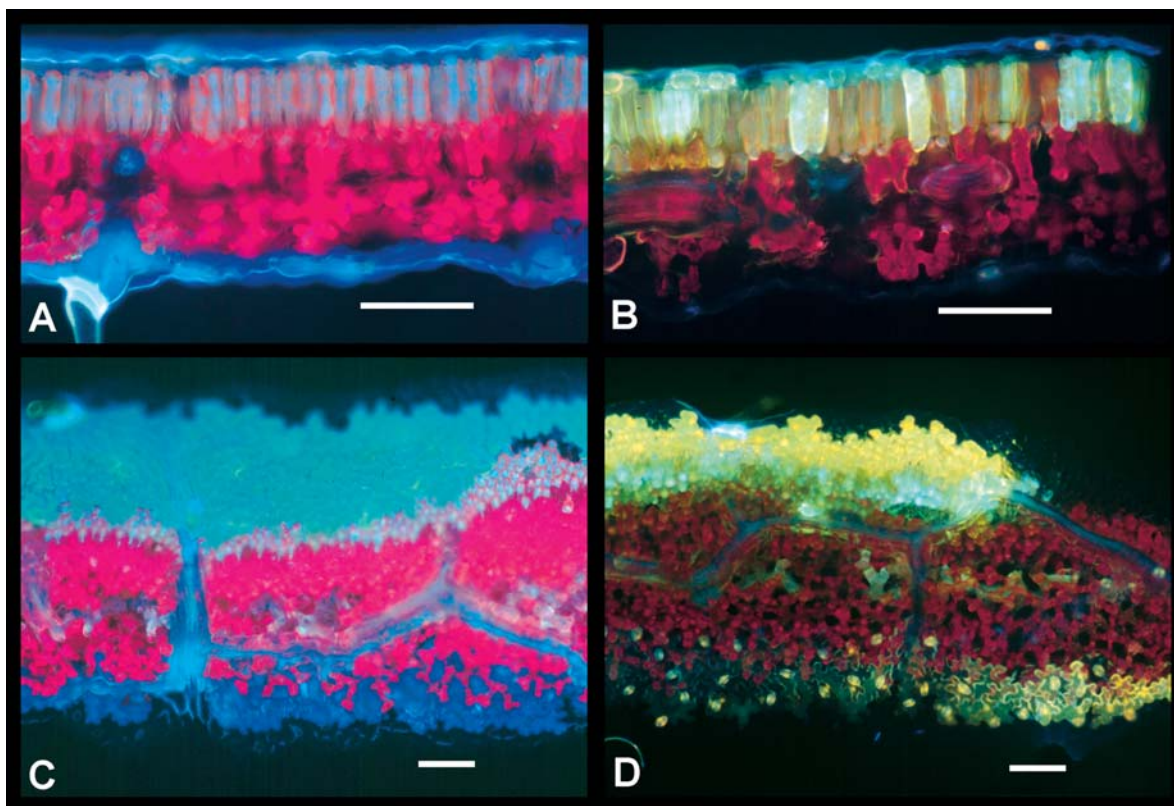


Figure 1. Freshly prepared paradermal (para-sec) or transversely sectioned (X-sec) soybean leaves fumed with ammonia (NH_3) or treated with NP and

cross sections and paradermal sections obtained by cutting the leaf at a very shallow angle with respect to the epidermis (Fig. 1). The flavonoids in normally developing soybean leaves are almost exclusively flavonol glycosides, which are restricted to the epidermis (40) and exhibit distinctive yellow-orange fluorescence with NP (48). Fluorescence of upper epidermal guard and pavement cells was of similar intensity, but lower epidermal guard cells fluoresced more intensely relative to the neighboring

epidermal cells (Fig. 1d). HCA exhibit intense blue to blue-white fluorescence with both NP and NH_3 and are found in both epidermis and mesophyll (Fig. 1a,b). HCA fluorescence with NH_3 was greater in upper epidermal tissues (Fig. 1c) as compared with lower epidermal tissue, consistent with results obtained with NP. We conclude that expression of both HCA and flavonols is characteristic of upper epidermal pavement cells, whereas flavonol accumulation is restricted to stoma in the lower epidermis.

Although the adaxial leaf surface receives direct sunlight most of the time, the lower surface may become exposed by tropic leaf movements or wind-induced leaf flutter. Because much of the terrestrial UV-B is obtained by indirect whole-sky irradiance, tissues can receive a substantial UV dose even without direct insolation. The relatively high flavonoid concentration in the abaxial guard cells may function to protect guard cells against UV in such instances. Our results may also explain why estimation of UV penetration into field-grown soybean leaves by methods relying on chlorophyll fluorescence is suitable only for the lower epidermis (49).

Decreased stomatal density

Reports of UV-B effects on stomatal density are scarce. Dai *et al.* (29) reported that elevated UV-B significantly reduced stomatal density and stomatal opening in rice. Stomatal density was unchanged in pea plants by UV-B due to general decline in leaf cell numbers and cell division, although UV-B decreased stomatal conductance and leaf area (31).

Our experiments have shown that UV can reduce both stomatal density and conductance in soybean (Fig. 2). Soybean plants (cvs. Essex and Williams) were grown in chambers under high

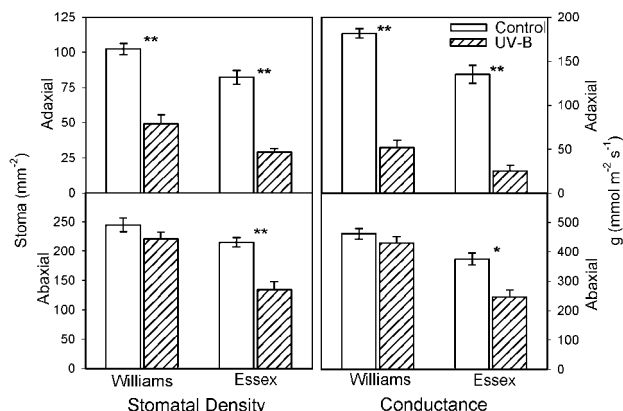


Figure 2. Stomatal density (left) and stomatal conductance (right) of adaxial (upper) and abaxial (lower) leaf surfaces of soybean (cvs. Williams and Essex) grown under UVB-313 lamps (Q-Panel Co.) wrapped with either polyester film (–UV-B, open bars) or cellulose diacetate film (+UV-B, cross-hatched bars) against $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ background radiation delivered by low-pressure sodium lamps. Error bars are SE. * is $P < 0.01$, ** is $P < 0.001$ and no asterisk is $P > 0.05$.

background irradiance ($800 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetic photon flux delivered from low-pressure sodium lamps) with and without supplemental UV-B from UVB-313 fluorescent lamps (Q-Panel Co., Cleveland, OH) for 8 h about midday, essentially according to methods described elsewhere (50,51). Height of UV-B lamps was adjusted so that the plants received $15 \text{ kJ m}^{-2} \text{day}^{-1}$ of biologically effective radiation according to a generalized plant response function, corresponding to the expected clear sky dose at summer solstice simulating a 30% stratospheric ozone depletion at Beltsville, MD (7,52). Both adaxial stomatal density and stomatal conductance were reduced (Fig. 2).

In similarly conducted glasshouse studies, UV-B equivalent to that expected with 25% ozone depletion elicited similar reduction of both upper epidermal stomatal density and conductance, but the degree of the reduction was less. Measurements of instantaneous water-use efficiency and $\delta^{13}\text{C}$ discrimination demonstrated that plant development under supplemental UV-B led to increased water-use efficiency (unpublished results). Taken together, the results were consistent with UV-B-increased water-use efficiency through reductions in stomatal density, although the degree of these responses was cultivar dependent. Although these studies did not eliminate the role that other photomorphogenic responses might play in affecting gas exchange, they did point to the potential importance of such mechanisms.

CONCLUSIONS

Our working hypothesis is that in some species, UV-B serves as a signal inducing specific photomorphogenic responses, leading to increased water-use efficiency. Because different species have evolved different adaptive mechanisms to UV-B and drought stress, a plant might exhibit one or all the discussed mechanisms, an incentive for the study of such developmental responses in species adapted to different environments. At the least, the soybean system warrants further study from a practical standpoint. Identification of traits associated with specific mechanisms through which UV photomorphogenic responses enhance water-use efficiency would benefit current breeding programs.

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